Molecular Therapy
Nucleic Acids
Original Article

Associations of lncRNA *H19* Polymorphisms at MicroRNA Binding Sites with Glioma Susceptibility and Prognosis

Yujiao Deng,[1,2,4] Linghui Zhou,[1,2,4] Jia Yao,[1] Yu Liu,[1] Yi Zheng,[1,2] Si Yang,[1,2] Ying Wu,[1,2] Na Li,[1,2] Peng Xu,[2] Lijuan Lyu,[2] Dai Zhang,[2] Jun Lyu,[3] and Zhijun Dai[1,2]

[1]Department of Breast Surgery, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, China; [2]Department of Oncology, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710004, China; [3]Department of Clinical Research, The First Affiliated Hospital of Jinan University, Guangzhou 510632, Guangzhou Province, China

Glioma is the most common tumor of the central nervous system; variation in susceptibility and prognosis worldwide suggests that there are molecular and genetic differences among individuals. The *H19* gene plays a dual role in carcinogenesis. In this study, associations between *H19* polymorphisms and susceptibility as well as prognosis in glioma were evaluated. In total, 605 patients with glioma and 1,300 cancer-free subjects were enrolled in the study. Individuals with the rs3741219 A>G allele were less likely to develop glioma (relative risk [RR] = 0.54, 95% confidence interval [95% CI] = 0.45–0.63, $p < 0.001$), whereas rs217727 G>A and rs2839698 G>A genotypes were not associated with glioma risk. The associations between *H19* polymorphisms and prognosis were assessed, including overall survival and progression-free survival. Three focused *H19* polymorphisms did not show a significant effect on survival. Further analysis based on false-positive report probability validated these significant results. In the haplotype analysis, individuals with the $G_{rs217727}A_{rs2839698}G_{rs3741219}$ haplotype were less likely to develop glioma (odds ratio [OR] = 0.33, 95% CI = 0.23–0.46, $p = 0.02$). Overall, carriers of the rs3741219 AG or GG genotype of *H19* have a decreased susceptibility to glioma, but polymorphisms in this gene are not related to prognosis.

INTRODUCTION

Glioma, which arises from glial or precursor cells, is one of the most common and highly fatal brain tumors.[1,2] According to the biological and clinical characteristics, glioma is classified into four World Health Organization (WHO) grades (I–IV).[3] Despite substantial advances in multimodal treatments, including surgery, radiotherapy, and chemotherapy, the overall survival (OS) of patients with glioma remains poor,[4] and it varies by race or ethnicity.[5] The risk factors of glioma include allergies/atopic disease, genetic factors, and ionizing radiation, among others.[6,7]

Glioma is a typical example of disease, for which molecular and genetic diagnoses affect patient treatment.[8] Long non-coding RNAs (lncRNAs) play crucial roles in glioma occurrence and development, including tumorigenesis, angiogenesis, and migration.[9,10] Single-nucleotide polymorphisms (SNPs) contribute to altering the binding of transcription factors, RNA splicers, and gene promoters, thereby regulating gene function.[11]

H19, a paternally inherited gene located on chromosome 11p15.5, is tightly linked to the insulin-like growth factor 2 (*IGF2*) gene.[12] The imprinted gene *H19* does not encode any protein, but it encodes a capped, spliced, polyadenylated, and oncofetal 2.7-kb RNA that is downregulated postnatally.[11] Genome-wide association studies have identified inherited risk factors as a feature of brain cancer genetics, and they have indicated that SNPs are usually present in patients with glioma.[13] The effect of *H19* on carcinogenesis is controversial. Jiang et al.[10] found that *H19* promotes the invasion and tumorigenicity of glioblastoma cells and could be a therapeutic target for glioblastoma. Three SNPs (rs4930101, rs11042170, and rs27359703) in *H19* remarkably increase colorectal cancer susceptibility.[14] The rs2071095 in *H19* is linked to the risk of breast cancer.[15] The rs2839698 might predict the risk and prognosis of hepatocellular cancer.[16] In addition, the rs3024270 GG genotype might increase neuroblastoma risk in female Chinese children.[17] In contrast, the rs2839698 TC genotype of *H19* significantly decreases the risk of bladder cancer.[11] Another six-center case-control study stated that none of three SNPs (rs2839698 G>A, rs3024270 C>G, rs217727 G>A) was relevant to the neuroblastoma susceptibility.[18]

However, the association between *H19* SNPs and glioma has not been examined to date. Hence, this hypothesis-driven case-control study aimed to investigate the associations between three SNPs

Received 29 October 2019; accepted 5 February 2020;
https://doi.org/10.1016/j.omtn.2020.02.003.

[4]These authors contributed equally to this work.

Correspondence: Zhijun Dai, Department of Breast Surgery, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, China.
E-mail: dzj0911@126.com
Correspondence: Jun Lyu, Department of Clinical Research, The First Affiliated Hospital of Jinan University, Guangzhou 510632, Guangzhou Province, China.
E-mail: lyujun2019@163.com

Table 1. Genotype Frequencies of *H19* Polymorphisms in Cases and Controls

Model	Genotype	Control (n, %)	Case (n, %)	OR[a] (95% CI)	p Value[a]
rs217727 HWE: p = 0.80					
Co-dominant	GG	557 (42.8)	254 (42.0)	1.00 (reference)	
Heterozygote	GA	591 (45.5)	278 (45.9)	1.03 (0.84–1.27)	0.77
Homozygote	AA	152 (11.7)	73 (12.1)	1.05 (0.77–1.44)	0.75
Dominant	GG	557 (42.8)	254 (42.0)	1.00 (reference)	
	GA+AA	743 (57.2)	351 (58.0)	1.04 (0.85–1.26)	0.72
Recessive	GG+GA	1,148 (88.3)	532 (87.9)	1.00 (reference)	
	AA	152 (11.7)	73 (12.1)	1.04 (0.77–1.40)	0.81
Overdominant	GG+AA	709 (54.5)	327 (54.1)	1.00 (reference)	
	GA	591 (45.5)	278 (45.9)	1.02 (0.84–1.24)	0.84
Allele	G	1,705 (65.6)	786 (65.0)	1.00 (reference)	
	A	895 (34.4)	424 (35.0)	1.03 (0.89–1.19)	0.71
rs2839698 HWE: p = 0.06					
Co-dominant	GG	675 (51.9)	311 (51.3)	1.00 (reference)	
Heterozygote	GA	504 (38.8)	240 (39.7)	1.03 (0.84–1.27)	0.75
Homozygote	AA	121 (9.3)	54 (9.0)	0.97 (0.68–1.37)	0.86
Dominant	GG	675 (51.9)	311 (51.3)	1.00 (reference)	
	GA+AA	625 (48.1)	294 (48.7)	1.02 (0.84–1.24)	0.83
Recessive	GG+GA	1,179 (90.7)	551 (91.0)	1.00 (reference)	
	AA	121 (9.3)	54 (9.0)	0.96 (0.68–1.34)	0.79
Overdominant	GG+AA	796 (61.2)	365 (60.3)	1.00 (reference)	
	GA	504 (38.8)	240 (39.7)	1.04 (0.85–1.27)	0.71
Allele	G	1,854 (71.3)	862 (71.2)	1.00 (reference)	
	A	746 (28.7)	348 (28.8)	1.00 (0.86–1.17)	0.97
rs3741219 HWE: p = 0.096					
Co-dominant	AA	651 (50.1)	439 (72.56)	1.00 (reference)	
Heterozygote	GA	520 (40.0)	107 (17.69)	0.31 (0.24–0.39)	<0.0001*
Homozygote	GG	129 (9.9)	59 (9.75)	0.68 (0.49–0.94)	0.02*
Dominant	AA	651 (50.1)	439 (72.56)	1.00 (reference)	
	GA+GG	649 (49.9)	166 (27.44)	0.38 (0.31–0.47)	<0.0001*
Recessive	AA+GA	1,171 (90.1)	546 (90.25)	1.00 (reference)	
	GG	129 (9.9)	59 (9.75)	0.98 (0.71–1.36)	0.91
Overdominant	AA+GG	780 (60.0)	498 (82.31)	1.00 (reference)	
	GA	520 (40.0)	107 (17.69)	0.32 (0.25–0.41)	<0.0001*
Allele	A	1,822 (70.1)	985 (81.40)	1.00 (reference)	
	G	778 (29.9)	225 (18.60)	0.54 (0.45–0.63)	<0.0001*

*$p \leq 0.05$ indicates statistical significance. OR = 1 (reference compared with other genotypes). HWE, Hardy-Weinberg equilibrium; OR, odds ratio; CI, confidence interval.
[a]Adjusted for age and sex.

(rs217727 G>A, rs2839698 G>A, and rs3741219 A>G) in *H19* and glioma susceptibility and prognosis.

RESULTS

Characteristics of Study Subjects

All 605 patients with glioma (270 females and 335 males) included in this study were of Han Chinese ethnicity. The survival time for patients ranged from 2 to 44 months, with a median survival time of 11 months. In addition, the clinical characteristics included sex, age, WHO grade, history of surgery, radiotherapy, and chemotherapy (Table S1). Patients were divided into two groups according to WHO grade: 382 patients (63.1%) with grades I–II, and 223 patients (36.9%) with grades III–IV. A total of 416 patients (68.8%) underwent gross total resection (GTR), and 189 patients (31.2%) underwent subtotal

Table 2. Associations between *H19* Gene Polymorphisms and Clinical Characteristics of Glioma Patients

Characteristics	Genotype Distributions			
	AA	Aa	aa	Aa + aa
rs217727				
Age				
<40/≥40	99/155	136/142	32/41	168/183
OR (95% CI)	Ref.	0.67 (0.47–0.94)	0.82 (0.48–1.39)	0.70 (0.50–0.96)
p value[a]		0.021*	0.456	0.03*
Sex				
Male/female	138/116	155/123	42/31	197/154
OR (95% CI)	Ref.	0.94 (0.67–1.33)	0.88 (0.52–1.48)	0.93(0.67–1.29)
p value[a]		0.741	0.628	0.661
WHO grade				
I+II/III+IV	155/99	184/94	43/30	227/124
OR (95% CI)	Ref.	0.80 (0.56–1.14)	1.09 (0.64–1.85)	0.86 (0.61–1.19)
p value[a]		0.216	0.744	0.359
rs2839698				
Age				
<40/≥40	130/181	118/122	18/36	136/158
OR (95% CI)	Ref.	0.75 (0.53–1.05)	1.44 (0.80–2.71)	0.84 (0.61–1.16)
p value[a]		0.091	0.237	0.285
Sex				
Male/female	171/140	134/106	31/23	165/129
OR (95% CI)	Ref.	0.96 (0.68–1.35)	0.90 (0.50–1.61)	0.95 (0.69–1.31)
p value[a]		0.816	0.726	0.751
WHO grade				
I+II/III+IV	202/109	152/88	28/26	180/114
OR (95% CI)	Ref.	1.07 (0.75–1.52)	1.71 (0.95–3.07)	1.17 (0.84–1.63)
p value[a]		0.715	0.070	0.358
rs3741219				
Age				
<40/≥40	197/242	46/61	24/35	70/96
OR (95% CI)	Ref.	1.08 (0.71–1.66)	1.19 (0.69–2.08)	1.12 (0.78–1.60)
p value[a]		0.725	0.543	0.550
Sex				
Male/female	237/202	60/47	38/21	98/68
OR (95% CI)	Ref.	0.92 (0.60–1.40)	0.65 (0.36–1.13)	0.81(0.57–1.17)
p value[a]		0.697	0.133	0.265
WHO grade				
I+II/III+IV	281/158	62/45	39/20	101/65
OR (95% CI)	Ref.	1.29 (0.84–1.98)	0.91 (0.51–1.60)	1.44 (0.79–1.65)
p value[a]		0.245	0.753	0.472

A, wild allele; a, variant allele. OR, odds ratio; CI, confidence interval; Ref., reference; WHO, World Health Organization. *$p \leq 0.05$.

[a]Univariate logistic regression analysis for the distributions of genotype frequencies. $p \leq 0.05$ indicates statistical significance.

resection (STR) or near-total resection (NTR). Except for 60 patients, all subjects received radiotherapy. Among these patients, 162 patients (26.8%) underwent conformal radiotherapy and 383 patients (63.3%) underwent gamma knife therapy. In total, 250 patients (41.3%) received chemotherapy (124 patients received platinum-based agents, 52 patients received temozolomide, and 74 patients received nimustine), and 355 patients did not receive any chemotherapy. The age and sex distributions in the case and control groups were balanced (p = 0.688 and p = 0.534). Furthermore, there was no statistically significant difference in the average age between the case (40.71 ± 18.28 years) and control groups (41.68 ± 13.54 years) (p = 0.195).

Association between H19 Polymorphisms and Glioma Susceptibility

Table 1 presents the genotypes and allele frequencies of *H19* in the two groups and their associations with glioma susceptibility, adjusted for sex and age. The genotype frequency distributions of the three polymorphisms conformed to the Hardy-Weinberg equilibrium (HWE) (rs217727, p = 0.80; rs2839698, p = 0.06; rs3741219, p = 0.096).

We applied six genetic models to investigate the association between *H19* polymorphisms and glioma risk. All of the inheritance models indicated that rs217727 and rs2839698 were not associated with glioma susceptibility (Table 1). However, all inheritance models revealed that rs3741219 A>G was significantly associated with a decreased risk of glioma, except for the recessive model (heterozygote: GA versus AA, odds ratio [OR] = 0.31, 95% confidence interval [95% CI] = 0.24–0.39, p < 0.001; homozygote: GG versus AA, OR = 0.68, 95% CI = 0.49–0.94, p = 0.02; dominant: GA+GG versus AA, OR = 0.38, 95% CI = 0.31–0.47, p < 0.001; overdominant: GA versus AA+GG, OR = 0.32, 95% CI = 0.25–0.41, p < 0.001; allele: A versus G, OR = 0.54, 95% CI = 0.45–0.63, p < 0.001).

Associations between H19 Gene Polymorphisms and Clinical Characteristics

We further analyzed the associations between clinical features in patients with glioma and *H19* polymorphisms, stratified by age, sex, tumor sites, and WHO grade (Table 2). This analysis revealed that the GA/AA and AA genotypes of *H19* rs217727 in patients aged ≥40 years were less frequent than the GG genotype in patients aged <40 years (GA+AA versus GG: OR = 0.70, 95% CI = 0.50–0.96, p = 0.03; AA versus GG: OR = 0.67, 95% CI = 0.47–0.94, p = 0.02). For rs2839698 and rs3741219, no significant association between polymorphisms and clinical characteristics was observed.

False-Positive Report Probability (FPRP) Results

We preset 0.2 as the FPRP threshold. As shown in Table S2, at the prior probability of 0.01, all of the significant findings for the *H19* rs3741219 A>G polymorphism (GA versus AA, GG versus AA, GA+GG versus AA, GA versus AA+GG) remained noteworthy. Moreover, the association with the *H19* rs3741219 allele variation (A>G) was also noteworthy, with a statistical power of 0.709 and a FPRP value of 0.001.

Table 3. The Haplotype Analysis of Three *H19* Gene Polymorphisms (rs217727 G>A, rs2839698 G>A, and rs3741219 A>G)

Haplotypes	Case (%)	Control (%)	OR (95% CI)	p Value
$G_{rs217727}G_{rs2839698}A_{rs3741219}$	189 (31.3)	463 (35.58)	Ref.	
$A_{rs217727}A_{rs2839698}A_{rs3741219}$	3 (0.4)	0 (0.00)	NA	NA
$A_{rs217727}A_{rs2839698}G_{rs3741219}$	1 (0.1)	0 (0.00)	NA	NA
$A_{rs217727}G_{rs2839698}A_{rs3741219}$	178 (29.5)	445 (34.22)	0.98 (0.77–1.25)	0.87
$A_{rs217727}G_{rs2839698}G_{rs3741219}$	30 (5)	2 (0.16)	NA	NA
$G_{rs217727}A_{rs2839698}A_{rs3741219}$	122 (20.2)	2 (0.18)	NA	NA
$G_{rs217727}A_{rs2839698}G_{rs3741219}$	49 (8.1)	369 (28.42)	0.33 (0.23–0.46)	<0.0001*
$G_{rs217727}G_{rs2839698}G_{rs3741219}$	33 (5.4)	19 (1.44)	NA	NA

OR, odds ratio; CI, confidence interval; Ref., reference; NA, not applicable. *$p \leq 0.05$.

Haplotype Analysis

As shown in Table 3, we conducted a haplotype analysis to evaluate the joint action of three *H19* SNPs. The $G_{rs217727}A_{rs2839698}G_{rs3741219}$ haplotype was associated with a reduced risk of glioma, compared with the wild-type haplotype $G_{rs217727}G_{rs2839698}A_{rs3741219}$ (OR = 0.33, 95% CI = 0.23–0.46, p = 0.02).

Association of H19 Polymorphisms with Glioma Prognosis

We investigated the association between the three SNPs as well as other potential factors and glioma prognosis by univariate Cox analyses. The subgroup analysis was stratified by age, sex, surgery, WHO grade, and history of chemotherapy and radiotherapy. By univariate Cox analyses, no significant association was observed between three *H19* polymorphisms and glioma prognosis (OS and progression-free survival [PFS]). However, three factors, including age, chemotherapy, and surgery, were identified as independent prognostic factors of glioma (Tables 4 and 5).

Furthermore, we performed a multivariate Cox analysis of the three factors identified above. As presented in Figures 1 and 2, patients with glioma aged ≥40 years showed worse OS and PFS (OS: hazard ratio [HR] = 1.21, 95% CI = 1.02–1.44, p = 0.029; PFS: HR = 1.21, 95% CI = 1.02–1.43, p = 0.03) compared with those of younger patients. In addition, compared with patients who underwent STR or NTR, those who underwent GTR showed better OS and PFS (OS: HR = 0.62, 95% CI = 0.51–0.75, p < 0.001; PFS: HR = 0.61, 95% CI = 0.51–0.74, p < 0.001). Furthermore, there was a sizeable difference in prognosis depending on various chemotherapy regimens. Compared with patients who received no chemotherapy as a reference, patients treated with temozolomide or nimustine presented improved OS (temozolomide: HR = 0.36, 95% CI = 0.24–0.52, p < 0.001; nimustine: HR = 0.74, 95% CI = 0.56–0.97, p = 0.030). As for PFS, patients who received temozolomide treatment had a longer PFS (HR = 0.38, 95% CI = 0.26–0.55, p < 0.001), whereas patients who received platinum or nimustine-based treatment did not show significantly better therapeutic effects. In the Kaplan-Meier and log-rank analyses (Figure 3), the three *H19* variants showed no association with glioma prognosis (OS: rs217727, p = 0.52; rs2839698, p = 0.99; rs3741219, p = 0.80; PFS: rs217727, p = 0.41; rs2839698, p = 0.88; rs3741219, p = 0.85).

DISCUSSION

Although the function of *H19* has been investigated in the past few years, its exact role in carcinogenesis is controversial. An association between *H19* polymorphisms and glioma occurrence or prognosis has never been reported. In our analysis, the role of *H19* polymorphisms in the regulation of glioma carcinogenesis was found to be complex. The initiation and development of glioma are influenced by both genetic and external factors. In this hospital-based study, the rs3741219 allele variation of *H19* was associated with a decreased risk of glioma occurrence. In addition, the mutant $G_{rs217727}A_{rs2839698}G_{rs3741219}$ haplotype of *H19* could substantially reduce the risk of glioma, mirroring that the rs3741219 A>G variant might play a beneficial role in glioma prevention.

The *H19* gene, which encodes a 2.3-kb lncRNA, is essential in embryonic development. Additionally, its expression is generally decreased after birth, with expression restricted to the cardiac and skeletal muscles. *H19* is an imprinted gene comprising five exons. Genomic imprinting is a gamete-specific inherited modification that determines allele-specific expression in somatic cells. In addition, loss of imprinting (LOI) in the gene might result in the development of some tumors, but not ubiquitously.[19] However, Uyeno et al.[12] suggested that LOI of *IGF2* but not *H19* is related to glioma development. Another study has indicated that c-Myc combines with the conserved E boxes near the imprinted control region of *H19*, inducing histone acetylation and transcriptional initiation, and increasing the expression of *H19*.[20] Structure determines function, and SNPs can affect promoter activity, mRNA stability, and translation efficiency, all of which, in turn, influence gene expression.[21] In addition, the mechanisms of the *H19* gene in cancer include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, genomic instability and mutation, and tumor-promoting inflammation, deregulating cellular energetics, and avoiding immune destruction.[22] The precise mechanisms deserve further investigation.

The rs217727 polymorphism is located in exon 5 of the *H19* gene. rs217727 is related to a significantly increased risk of non-small-cell lung cancer,[23] urothelial cell carcinoma,[24] bladder cancer,[25] breast cancer,[26] oral squamous cell carcinoma,[27] osteosarcoma,[28] and gastric cancer.[21] In contrast, another study has suggested that rs217727 polymorphism is related to a low risk of breast cancer.[28] It has also been reported that rs217727 C>T shows no association with the occurrence of breast cancer,[29] lung cancer,[30] and bladder cancer.[31] These results provide no consensus on the promoting or protective effect of rs217727 on cancer susceptibility. However, we found that the frequencies of GA+AA and AA genotypes of H19 rs217727 in patients aged ≥40 years were lower than those of the GG genotype in patients

Table 4. Analysis of *H19* Gene Polymorphisms and Clinical Features in Glioma Patient Overall Survival

Characteristics	Patients (n)	Events (n)	Rate (%)	Univariate Analysis	
				HR (95% CI)	p Value[a]
Age					
<40 years	267	229	85.77	Ref.	Ref.
≥40 years	338	310	91.72	1.20 (1.01–1.42)	0.039*
Sex					
Male	335	297	88.66	Ref.	Ref.
Female	270	242	89.63	1.08 (0.91–1.28)	0.355
WHO grade					
I–II	382	336	87.96	Ref.	Ref.
III–IV	223	206	92.38	1.18 (0.98–1.40)	0.063
Surgery					
STR and NTR	189	186	98.41	Ref.	Ref.
GTR	416	353	84.86	0.59 (0.49–0.71)	<0.001*
Chemotherapy					
No	355	333	93.80	Ref.	Ref.
Platinum	124	112	90.32	0.84 (0.68–1.04)	0.116
Temozolomide	52	30	57.69	0.32 (0.22–0.48)	<0.001*
Nimustine	74	64	86.49	0.645 (0.49–0.85)	0.001*
Radiotherapy					
No	60	49	81.67	Ref.	Ref.
Conformal Radiotherapy	162	133	82.10	1.08 (0.77–1.50)	0.622
Gamma knife	383	357	93.21	1.17 (0.86–1.58)	0.303
rs217727					
GG	254	226	88.98	Ref.	Ref.
GA	278	249	89.57	1.09 (0.83–1.44)	0.527
AA	73	64	87.67	1.14 (0.86–1.50)	0.357
rs2839698					
GG	311	281	90.35	Ref.	Ref.
GA	240	211	87.92	1.00 (0.73–1.36)	0.974
AA	54	47	87.04	0.97 (0.71–1.34)	0.869
rs3741219					
AA	439	391	89.07	Ref.	Ref.
GA	107	94	87.85	1.05 (0.84–1.31)	0.693
GG	59	54	91.53	1.09 (0.82–1.45)	0.564

OS, overall survival; HR, hazard ratio; CI, confidence interval; STR, subtotal resection; NTR, near-total resection; GTR, gross total resection; Ref., reference. *$p \leq 0.05$.
[a]Cox's proportional hazard regression analysis for univariate analysis. $p \leq 0.05$ indicates statistical significance.

aged <40 years, indicating that rs217727 might play various roles in the development of glioma depending on age.

In the present study, both *H19* rs217727 and rs2839698 showed no significant association with glioma susceptibility. Previous studies have reported that *H19* rs2839698 is not associated with the risk of developing non-small-cell lung cancer,[23] oral squamous cell carcinoma,[27] and breast cancer.[32] Another study revealed that heterozygotes of *H19* rs2839698 are more susceptible to hepatocellular carcinoma[33] and breast cancer.[26] rs2839698 is located within the 3′ untranslated region of the *H19* gene. A study in the Netherlands indicated that the rs2839698 polymorphism is associated with a decreased risk of bladder cancer,[31] which might be explained by variation in the exon. Exonic regions are related to the conserved secondary structure of the transcript or its binding affinity with interacting elements.

In our analysis, *H19* rs3741219 was associated with a reduced risk of glioma. The rs3741219 A/G polymorphism is located within the first exon of *H19*. Mutant alleles or structural variations in genes are presumed to be important, whereas variants that drive cancers are not unique. Divergent variants in the same gene could produce tumors with different characteristics.[8] The specific region of *H19* that harbors rs3741219 encodes an antisense transcript named the *H19* opposite tumor suppressor (*HOTS*),[34] which is antisense to the *H19* transcript and is conserved in primates. The overexpression of *HOTS*, which is localized within the nucleus and nucleolus, could inhibit the growth of some tumors. The *H19* locus could encode a tumor suppressor protein.[35] However, there is no evidence for the coexpression of *HOTS* and *H19 in vivo*. This complexity of inheritance and translation may partially explain the differences between associations of *H19* SNPs with distinct types of cancers.[35]

Furthermore, according to Cox regression analysis, age, surgery type, and chemotherapy were independent prognostic risk factors for glioma. However, our data indicated that these three *H19* gene polymorphisms were not associated with glioma prognosis. A previous study has reported that rs2839698 is related to a poor prognosis in hepatocellular cancer.[16] However, post-operative patients with gastric adenocarcinoma harboring the *H19* rs2839698 GA genotype had an improved prognosis. Further studies of the association between H19 gene polymorphisms and cancer prognosis are necessary to verify the results.

Using lncRNASNP2 (http://bioinfo.life.hust.edu.cn/lncRNASNP/), we found that the *H19* rs217727 G>A SNP may affect microRNA (miRNA)-lncRNA interactions, resulting in gains and losses of miRNA target sites, forming hsa-miR-4804-5p and hsa-miR-8071, and destroying hsa-miR-3960 and hsa-miR-8071 binding sites on *H19*. The rs3741219 A>G polymorphism causes the gain of miRNA target sites (including hsa-miR-3187-5p, hsa-miR-1285-3p, hsa-miR-6860, and hsa-miR-612), as well as miRNA target losses (including hsa-miR-4486, hsa-miR-24-1-5p, and hsa-miR-566). A similar genotype-phenotype association was also observed for rs3741219 A>G. This indicated that the *H19* rs3741219 A>G SNP creates hsa-miR-1539, hsa-miR-3193, and hsa-miR-146b-3p and damages hsa-miR-1914-5p, hsa-miR-6811-3p, and hsa-miR-6514-3p miRNA binding sites. The specific mechanisms underlying these effects require further investigations.

Table 5. Analysis of *H19* Gene Polymorphisms and Clinical Features in Glioma Patient Progression-Free Survival

Characteristics	Patients (n)	Events (n)	Rate (%)	Univariate Analysis	
				HR (95% CI)	p Value[a]
Age					
<40 years	267	239	89.51	Ref.	Ref.
≥40 years	338	324	95.86	1.19 (1.00–1.40)	0.047*
Sex					
Male	335	310	92.54	Ref.	Ref.
Female	270	253	93.70	1.1 (0.93–1.30)	0.263
WHO grade					
I–II	382	353	92.41	Ref.	Ref.
III–IV	223	210	94.17	1.15 (0.97–1.36)	0.116
Surgery					
STR and NTR	189	183	96.83	Ref.	Ref.
GTR	416	380	91.35	0.58 (0.48–0.69)	<0.001*
Chemotherapy					
No	355	351	98.87	Ref.	Ref.
Platinum	124	116	93.55	0.99 (0.80–1.22)	0.916
Temozolomide	52	32	61.54	0.35 (0.24–0.50)	<0.001*
Nimustine	74	64	86.49	0.73 (0.56–0.96)	0.022*
Radiotherapy					
No	60	55	91.67	Ref.	Ref.
Conformal radiotherapy	162	137	84.57	1.13 (0.83–1.56)	0.436
Gamma knife	383	371	96.87	1.21 (0.91–1.60)	0.199
rs217727					
GG	254	234	92.13	Ref.	Ref.
GA	278	261	93.88	1.00 (0.76–1.30)	0.973
AA	73	68	93.15	1.11 (0.85–1.46)	0.429
rs2839698					
GG	311	294	94.53	Ref.	Ref.
GA	240	219	91.25	1.04 (0.77–1.40)	0.797
AA	54	50	92.59	0.98 (0.72–1.34)	0.906
rs3741219					
AA	439	408	92.94	Ref.	Ref.
GA	107	101	94.39	1.01 (0.81–1.25)	0.940
GG	59	54	91.53	1.08 (0.81–1.44)	0.595

PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; STR, subtotal resection; NTR, near-total resection; GTR, gross total resection; Ref., reference. *$p \leq 0.05$.

[a]Cox's proportional hazard regression analysis for univariate analysis. $p \leq 0.05$ indicates statistical significance.

This study investigated the effect of *H19* gene polymorphisms on susceptibility and prognosis of glioma. However, additional studies are needed to verify these results and to address several limitations of our study. The effect of environmental factors on glioma risk might be underestimated, owing to a lack of exposure information. In addition, heterogeneity among individuals in various factors is inevitable. All participants were from northwestern China and were of Han ethnicity, resulting in selection bias. Despite these limitations, our study included the largest sample of patients with glioma to date. Additionally, to our knowledge, this is the first study focused on the association of H19 gene polymorphisms with glioma susceptibility and prognosis.

Taken together, the present data suggest that rs2839698 G>A in the *H19* gene is associated with a decreased glioma risk. Additionally, patients carrying the $G_{rs217727}A_{rs2839698}G_{rs3741219}$ haplotype were less prone to develop glioma. Our results mirror the complexity of *H19* functions in glioma initiation and development.

MATERIALS AND METHODS

Study Population

In total, 1,905 participants of Han Chinese ethnicity (605 patients with glioma and 1,300 controls) were consecutively enrolled in the Department of Neurosurgery at Tangdu Hospital, The Second Affiliated Hospital of The Fourth Military Medical University (Xi'an, China), from September 2010 to May 2014. All patients were pathologically diagnosed with glioma. Furthermore, the patients were not treated with chemotherapy or radiotherapy before surgery. Healthy controls, without a history of malignancies and underlying disease, were recruited among participants who underwent routine examinations in the same period and were matched with the glioma cases in terms of sex and age. The basic characteristics of the participants, including age, sex, ethnicity, WHO grade, surgery, chemotherapy strategy, and radiotherapy, were collected from medical records or self-administered questionnaires. Furthermore, a monthly follow-up was conducted by telephone and outpatient interviews. All patients provided written informed consent before participation. With respect to prognosis, the outcome indicators of death and disease progression were considered to explore the association between *H19* polymorphisms and OS as well as PFS. Patients with glioma included in this study were followed up for 44 months. This study was approved by the Institutional Review Board of The First Affiliated Hospital of Zhejiang University in Zhejiang Province (Hangzhou, China).

SNP Selection and Genotyping

The NCBI dbSNP database and the online tool software SNPinfo were used to select candidate SNPs. Three widely researched SNPs (rs217727 G>A, rs2839698 G>A, and rs3741219 A>G) in the *H19* gene were investigated. Peripheral blood was drawn from the participants and stored at −80°C in EDTA tubes for DNA extraction and genotyping. Genomic DNA was extracted using a universal genomic DNA extraction kit (Takara, Kyoto, Japan).[36] DNA concentrations were measured by spectrophotometry (DU530 UV/VIS spectrophotometer, Beckman Coulter, Fullerton, CA, USA). Sequenom MassARRAY assay design 3.0 (Sequenom, San Diego, CA, USA) was used for designing the multiplexed SNP MassEXTEND assay. Genotyping of *H19* polymorphisms was performed using the Sequenom MassARRAY RS1000. Sequenom Typer 4.0 was used for data analysis. Investigators were blind to the case-control group information for samples.

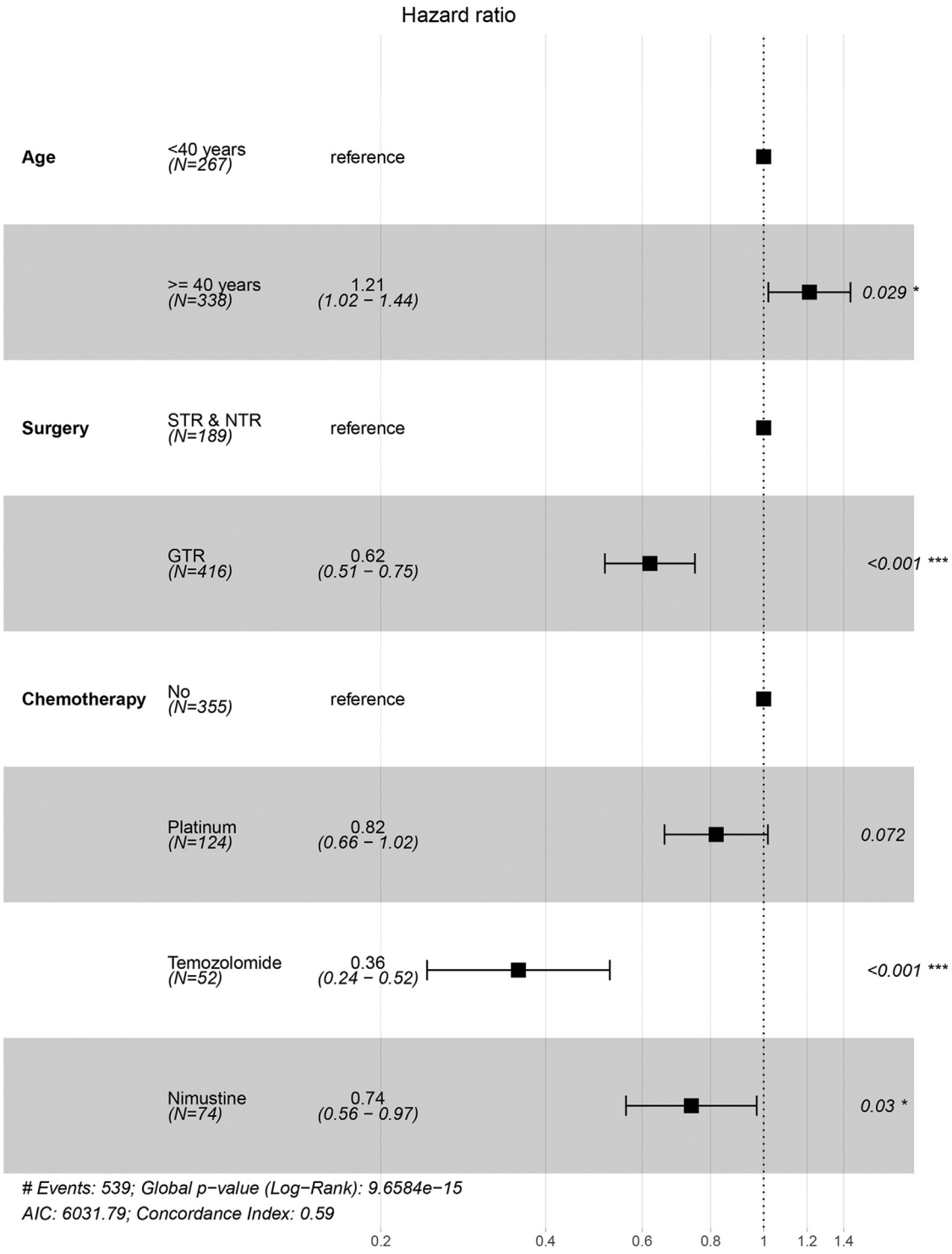

Figure 1. Forest Plots of Multivariate Cox Regression Analysis for Overall Survival

STR, subtotal resection; NTR, near-total resection; GTR, gross total resection.

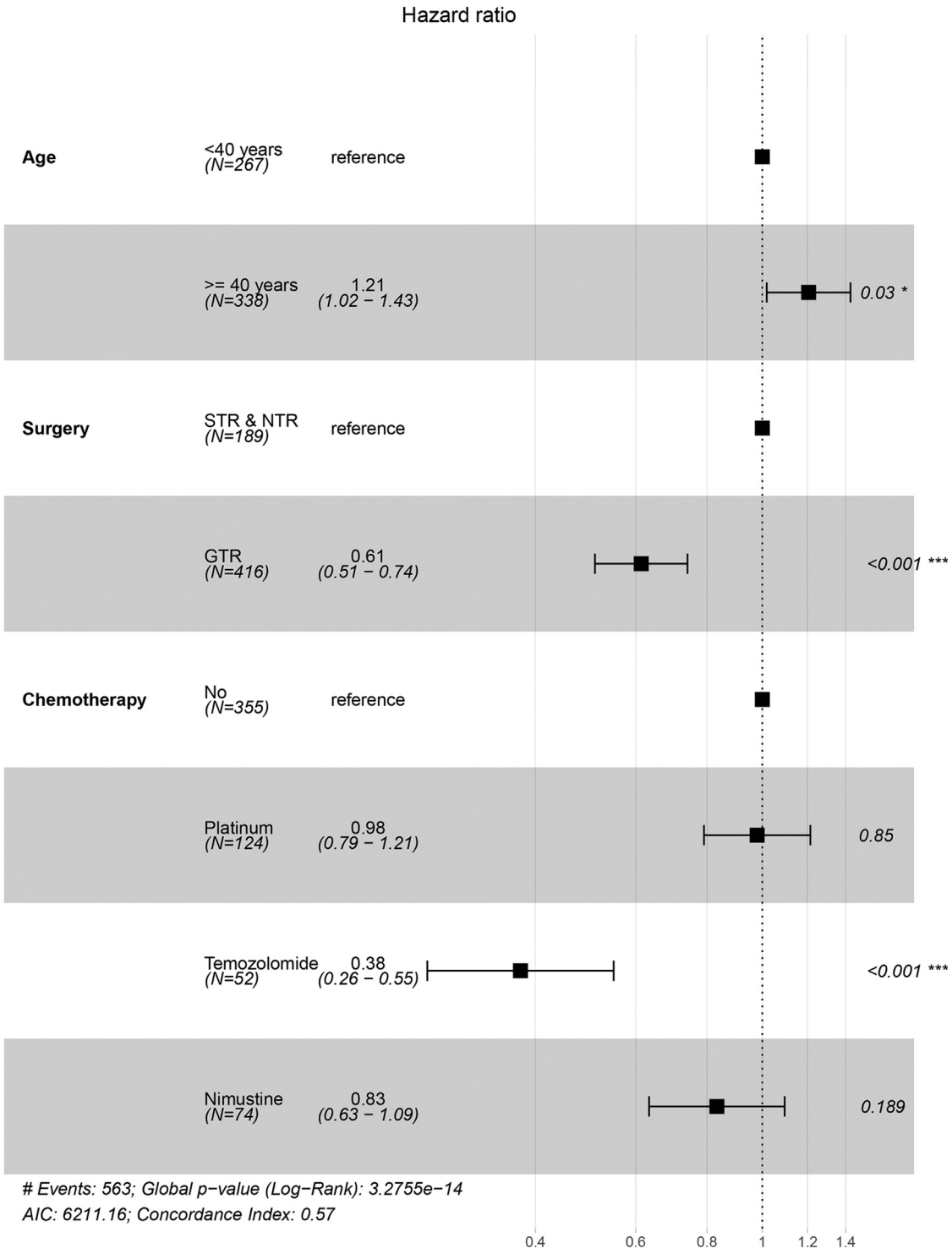

Figure 2. Forest Plots of Multivariate Cox Regression Analysis for Progression-Free Survival

STR, subtotal resection; NTR, near-total resection; GTR, gross total resection.

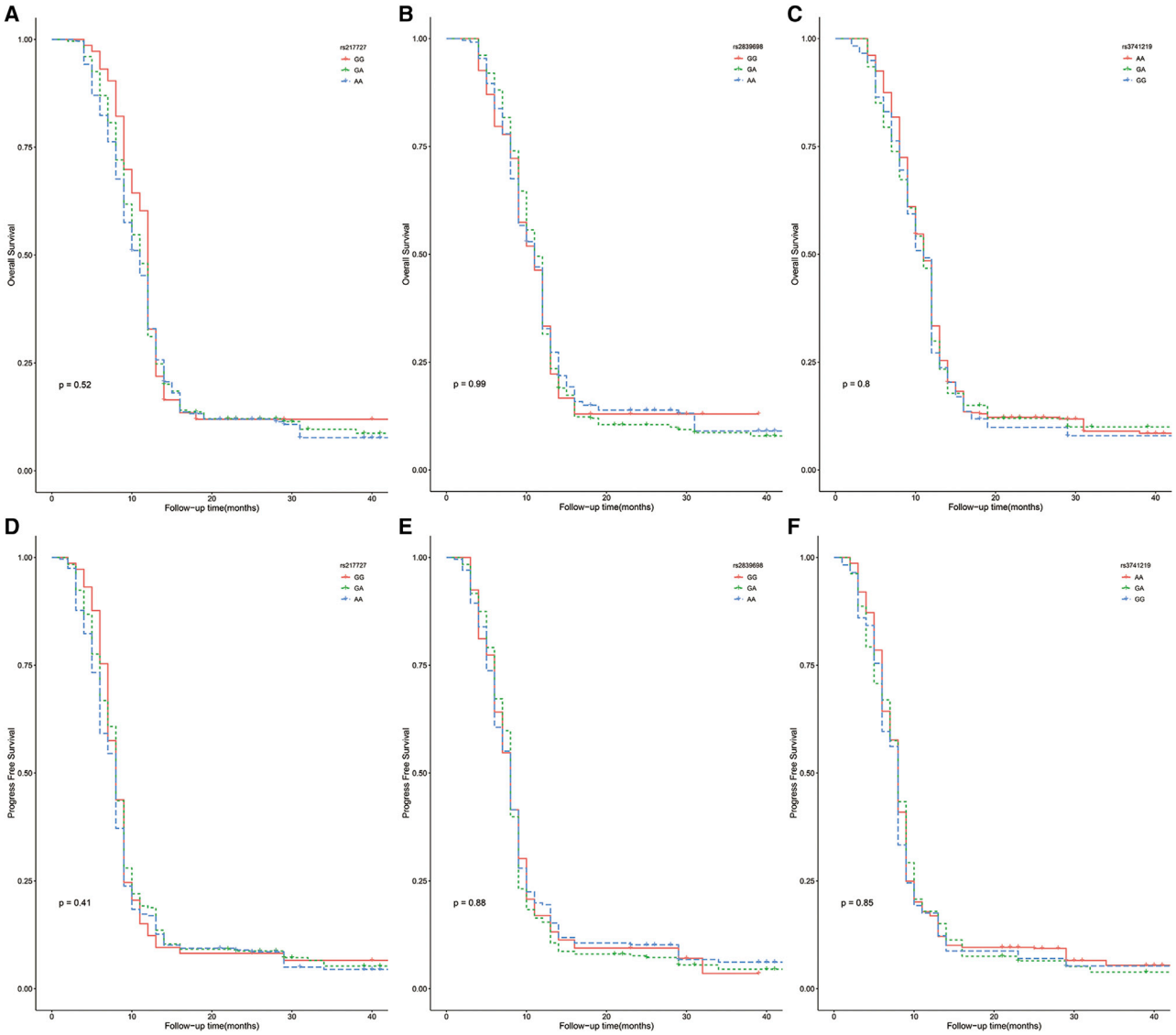

Figure 3. Kaplan-Meier Analysis of OS and PFS in Three Polymorphisms of the *H19* Gene

(A–F) OS of rs217727 (A); OS of rs2839698 (B); OS of rs3741219 (C); PFS of rs217727 (D); PFS of rs2839698 (E); and PFS of rs3741219 (F). OS, overall survival; PFS, progression-free survival.

All participants were genotyped successfully. The primers for the three SNPs are listed in Table S3.

Haplotype Analysis

A haplotype analysis was conducted using SHEsis (http://analysis.bio-x.cn/SHEsisMain.htm).[37] There is a lowest frequency threshold for haplotype analysis: any number in (0, 1) could be accepted. Haplotypes with a frequency less than this number will not be considered in analysis, and the default value is usually 0.03. Haplotypes with frequencies of less than 0.03 were not analyzed.

Statistical Analysis

R software (version 3.5.1) was used for statistical analyses, as described in our previous studies.[36,38–40] HWE was assessed by the goodness-of-fit χ^2 test. A χ^2 test or t test were used to compare the distributions of genotype frequencies between the case and control groups. A logistic regression analysis was conducted to evaluate the association between SNPs and glioma risk. ORs and their 95% CIs were calculated. To assess prognostic effects, univariate and multivariate Cox analyses were conducted, and HRs with 95% CIs were calculated. Stratified analyses were also conducted by age, sex, surgery, WHO grade, and history of chemotherapy and radiotherapy. Moreover, the FPRP analysis was performed to verify the significant

results.[41,42] All statistical tests used were two-sided, with a significance threshold of $p < 0.05$.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.omtn.2020.02.003.

AUTHOR CONTRIBUTIONS

All authors read, critically reviewed, and approved the final manuscript. Z.D. and J.L. designed the research; Y.D., Y.Z., and J.Y. collected the data; L.Z. and S.Y. performed the statistical analysis; Y.W. and P.X. provided methodological support/advice; L.L., N.L, and D.Z. conducted the experiments; and Y.D. wrote the manuscript.

CONFLICT OF INTEREST

The authors declare no competing interests.

ACKNOWLEDGMENTS

We thank all members of our study team for their wholehearted cooperation and the study participants for their wonderful contribution.

REFERENCES

1. Ostrom, Q.T., Gittleman, H., Fulop, J., Liu, M., Blanda, R., Kromer, C., Wolinsky, Y., Kruchko, C., and Barnholtz-Sloan, J.S. (2015). CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2008–2012. Neuro-oncol. *17* (*Suppl 4*), iv1–iv62.
2. Amirian, E.S., Armstrong, G.N., Zhou, R., Lau, C.C., Claus, E.B., Barnholtz-Sloan, J.S., Il'yasova, D., Schildkraut, J., Ali-Osman, F., Sadetzki, S., et al. (2016). The Glioma International Case-Control Study: a report from the Genetic Epidemiology of Glioma International Consortium. Am. J. Epidemiol. *183*, 85–91.
3. Louis, D.N., Perry, A., Reifenberger, G., von Deimling, A., Figarella-Branger, D., Cavenee, W.K., Ohgaki, H., Wiestler, O.D., Kleihues, P., and Ellison, D.W. (2016). The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. *131*, 803–820.
4. Molinaro, A.M., Taylor, J.W., Wiencke, J.K., and Wrensch, M.R. (2019). Genetic and molecular epidemiology of adult diffuse glioma. Nat. Rev. Neurol. *15*, 405–417.
5. Ostrom, Q.T., Cote, D.J., Ascha, M., Kruchko, C., and Barnholtz-Sloan, J.S. (2018). Adult glioma incidence and survival by race or ethnicity in the United States from 2000 to 2014. JAMA Oncol. *4*, 1254–1262.
6. Bauchet, L., and Ostrom, Q.T. (2019). Epidemiology and molecular epidemiology. Neurosurg. Clin. N. Am. *30*, 1–16.
7. Wiedmann, M.K.H., Brunborg, C., Di Ieva, A., Lindemann, K., Johannesen, T.B., Vatten, L., Helseth, E., and Zwart, J.A. (2017). The impact of body mass index and height on the risk for glioblastoma and other glioma subgroups: a large prospective cohort study. Neuro-oncol. *19*, 976–985.
8. DeWeerdt, S. (2018). The genomics of brain cancer. Nature *561*, S54–S55.
9. Kiang, K.M., Zhang, X.Q., and Leung, G.K. (2015). Long non-coding RNAs: the key players in glioma pathogenesis. Cancers (Basel) *7*, 1406–1424.
10. Jiang, X., Yan, Y., Hu, M., Chen, X., Wang, Y., Dai, Y., Wu, D., Wang, Y., Zhuang, Z., and Xia, H. (2016). Increased level of H19 long noncoding RNA promotes invasion, angiogenesis, and stemness of glioblastoma cells. J. Neurosurg. *124*, 129–136.
11. Brannan, C.I., Dees, E.C., Ingram, R.S., and Tilghman, S.M. (1990). The product of the H19 gene may function as an RNA. Mol. Cell. Biol. *10*, 28–36.
12. Uyeno, S., Aoki, Y., Nata, M., Sagisaka, K., Kayama, T., Yoshimoto, T., and Ono, T. (1996). IGF2 but not H19 shows loss of imprinting in human glioma. Cancer Res. *56*, 5356–5359.
13. Jonsson, P., Lin, A.L., Young, R.J., DiStefano, N.M., Hyman, D.M., Li, B.T., Berger, M.F., Zehir, A., Ladanyi, M., Solit, D.B., et al. (2019). Genomic correlates of disease progression and treatment response in prospectively characterized gliomas. Clin. Cancer Res. *25*, 5537–5547.
14. Qin, W., Wang, X., Wang, Y., Li, Y., Chen, Q., Hu, X., Wu, Z., Zhao, P., Li, S., Zhao, H., et al. (2019). Functional polymorphisms of the lncRNA *H19* promoter region contribute to the cancer risk and clinical outcomes in advanced colorectal cancer. Cancer Cell Int. *19*, 215.
15. Cui, P., Zhao, Y., Chu, X., He, N., Zheng, H., Han, J., Song, F., and Chen, K. (2018). SNP rs2071095 in LincRNA H19 is associated with breast cancer risk. Breast Cancer Res. Treat. *171*, 161–171.
16. Yang, M.L., Huang, Z., Wang, Q., Chen, H.H., Ma, S.N., Wu, R., and Cai, W.S. (2018). The association of polymorphisms in lncRNA-*H19* with hepatocellular cancer risk and prognosis. Biosci. Rep. *38*, BSR20171652.
17. Hu, C., Yang, T., Pan, J., Zhang, J., Yang, J., He, J., and Zou, Y. (2019). Associations between *H19* polymorphisms and neuroblastoma risk in Chinese children. Biosci. Rep. *39*, BSR20181582.
18. Li, Y., Zhuo, Z.J., Zhou, H., Liu, J., Zhang, J., Cheng, J., Zhou, H., Li, S., Li, M., He, J., et al. (2019). *H19* gene polymorphisms and neuroblastoma susceptibility in Chinese children: a six-center case-control study. J. Cancer *10*, 6358–6363.
19. Monk, D., Mackay, D.J.G., Eggermann, T., Maher, E.R., and Riccio, A. (2019). Genomic imprinting disorders: lessons on how genome, epigenome and environment interact. Nat. Rev. Genet. *20*, 235–248.
20. Barsyte-Lovejoy, D., Lau, S.K., Boutros, P.C., Khosravi, F., Jurisica, I., Andrulis, I.L., Tsao, M.S., and Penn, L.Z. (2006). The c-Myc oncogene directly induces the H19 noncoding RNA by allele-specific binding to potentiate tumorigenesis. Cancer Res. *66*, 5330–5337.
21. Yang, C., Tang, R., Ma, X., Wang, Y., Luo, D., Xu, Z., Zhu, Y., and Yang, L. (2015). Tag SNPs in long non-coding RNA H19 contribute to susceptibility to gastric cancer in the Chinese Han population. Oncotarget *6*, 15311–15320.
22. Lecerf, C., Le Bourhis, X., and Adriaenssens, E. (2019). The long non-coding RNA H19: an active player with multiple facets to sustain the hallmarks of cancer. Cell. Mol. Life Sci. *76*, 4673–4687.
23. Wang, G., Liu, Q., Cui, K., Ma, A., and Zhang, H. (2019). Association between H19 polymorphisms and NSCLC risk in a Chinese Population. J. BUON. *24*, 913–917.
24. Yang, P.J., Hsieh, M.J., Hung, T.W., Wang, S.S., Chen, S.C., Lee, M.C., Yang, S.F., and Chou, Y.E. (2019). Effects of long noncoding RNA H19 polymorphisms on urothelial cell carcinoma development. Int. J. Environ. Res. Public Health *16*, 1322.
25. Li, Z., and Niu, Y. (2019). Association between lncRNA H19 (rs217727, rs2735971 and rs3024270) polymorphisms and the risk of bladder cancer in Chinese population. Minerva Urol. Nefrol. *71*, 161–167.
26. Safari, M.R., Mohammad Rezaei, F., Dehghan, A., Noroozi, R., Taheri, M., and Ghafouri-Fard, S. (2019). Genomic variants within the long non-coding RNA H19 confer risk of breast cancer in Iranian population. Gene *701*, 121–124.
27. Guo, Q.Y., Wang, H., and Wang, Y. (2017). lncRNA H19 polymorphisms associated with the risk of OSCC in Chinese population. Eur. Rev. Med. Pharmacol. Sci. *21*, 3770–3774.
28. He, T.D., Xu, D., Sui, T., Zhu, J.K., Wei, Z.X., and Wang, Y.M. (2017). Association between H19 polymorphisms and osteosarcoma risk. Eur. Rev. Med. Pharmacol. Sci. *21*, 3775–3780.
29. Abdollahzadeh, S., and Ghorbian, S. (2019). Association of the study between *LncRNA-H19* gene polymorphisms with the risk of breast cancer. J. Clin. Lab. Anal. *33*, e22826.
30. Yin, Z., Cui, Z., Li, H., Li, J., and Zhou, B. (2018). Polymorphisms in the H19 gene and the risk of lung cancer among female never smokers in Shenyang, China. BMC Cancer *18*, 893.
31. Verhaegh, G.W., Verkleij, L., Vermeulen, S.H., den Heijer, M., Witjes, J.A., and Kiemeney, L.A. (2008). Polymorphisms in the *H19* gene and the risk of bladder cancer. Eur. Urol. *54*, 1118–1126.
32. Lin, Y., Fu, F., Chen, Y., Qiu, W., Lin, S., Yang, P., Huang, M., and Wang, C. (2017). Genetic variants in long noncoding RNA *H19* contribute to the risk of breast cancer in a southeast China Han population. OncoTargets Ther. *10*, 4369–4378.

33. Wu, E.R., Chou, Y.E., Liu, Y.F., Hsueh, K.C., Lee, H.L., Yang, S.F., and Su, S.C. (2019). Association of lncRNA *H19* gene polymorphisms with the occurrence of hepatocellular carcinoma. Genes (Basel) *10*, 506.

34. Matouk, I., Raveh, E., Ohana, P., Lail, R.A., Gershtain, E., Gilon, M., De Groot, N., Czerniak, A., and Hochberg, A. (2013). The increasing complexity of the oncofetal *H19* gene locus: functional dissection and therapeutic intervention. Int. J. Mol. Sci. *14*, 4298–4316.

35. Onyango, P., and Feinberg, A.P. (2011). A nucleolar protein, *H19* opposite tumor suppressor (HOTS), is a tumor growth inhibitor encoded by a human imprinted *H19* antisense transcript. Proc. Natl. Acad. Sci. USA *108*, 16759–16764.

36. Zhou, L., Dong, S., Deng, Y., Yang, P., Zheng, Y., Yao, L., Zhang, M., Yang, S., Wu, Y., Zhai, Z., et al. (2019). GOLGA7 rs11337, a polymorphism at the microRNA binding site, is associated with glioma prognosis. Mol. Ther. Nucleic Acids *18*, 56–65.

37. Shi, Y.Y., and He, L. (2005). SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res. *15*, 97–98.

38. Yao, L., Zhou, L., Deng, Y., Zheng, Y., Yang, P., Wang, M., Dong, S., Hao, Q., Xu, P., Li, N., et al. (2019). Association between genetic polymorphisms in *TYMS* and glioma risk in Chinese patients: a case-control study. OncoTargets Ther. *12*, 8241–8247.

39. Wu, Y., Zhou, L., Deng, Y., Li, N., Yang, P., Dong, S., Yang, S., Zheng, Y., Yao, L., Zhang, M., et al. (2019). The polymorphisms (rs3213801 and rs5744533) of DNA polymerase kappa gene are not related with glioma risk and prognosis: A case-control study. Cancer Med. *8*, 7446–7453.

40. Deng, Y., Zhou, L., Li, N., Wang, M., Yao, L., Dong, S., Zhang, M., Yang, P., Hao, Q., Wu, Y., et al. (2019). Impact of four lncRNA polymorphisms (rs2151280, rs7763881, rs1136410, and rs3787016) on glioma risk and prognosis: a case-control study. Mol. Carcinog. *58*, 2218–2229.

41. He, J., Wang, M.Y., Qiu, L.X., Zhu, M.L., Shi, T.Y., Zhou, X.Y., Sun, M.H., Yang, Y.J., Wang, J.C., Jin, L., et al. (2013). Genetic variations of mTORC1 genes and risk of gastric cancer in an Eastern Chinese population. Mol. Carcinog. *52* (*Suppl 1*), E70–E79.

42. He, J., Zou, Y., Liu, X., Zhu, J., Zhang, J., Zhang, R., Yang, T., and Xia, H. (2018). Association of common genetic variants in pre-microRNAs and neuroblastoma susceptibility: a two-center study in Chinese children. Mol. Ther. Nucleic Acids *11*, 1–8.